



EDGEWOOD

CHEMICAL BIOLOGICAL CENTER

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**DEPARTMENT OF TRANSPORTATION
DERMAL TEST OF NEUTRALIZED GB HYDROLYSATE
IN RABBITS**

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

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PREFACE

The work described in this report was authorized under Project No. 8VEJWM. The work was started in July 2008 and completed in December 2008. Technical data/test results are recorded in Laboratory Notebook No. 08-0122 and will be stored in the Life Science Official archives and/or the U.S. Army Edgewood Chemical Biological Center (ECBC) Technical Library.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Academy Press, Washington D.C., 1996. These investigations were also performed in accordance with the requirements of AR 70-18 (Animal Welfare Act), Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs, and the Institutional Animal Care and Use Committee [(IACUC) ECBC], which oversees the use of laboratory animals by reviewing for approval all ECBC research protocols requiring laboratory animals. This project, assigned IACUC Protocol No.08-406, was approved on 5 September 2008.

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QUALITY ASSURANCE (U)

(U) This study, conducted as described in Protocol 08-406, was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792. The report was titled, "Department of Transportation Dermal Test of Neutralized GB Hydrolysate in Rabbits"(U). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

<u>Phase Inspected</u>	<u>Date</u>	<u>Reported</u>
Study Parameters and Exposure	16 Sep 08	16 Sep 08
Data and Final Report	22 Jan 09	22 Jan 09

(U) To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.


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DEPARTMENT OF TRANSPORTATION DERMAL TEST OF NEUTRALIZED GB HYDROLYSATE IN RABBITS

1. INTRODUCTION

The Assembled Chemical Weapons Alternatives Branch was tasked with the destruction of chemical weapons stockpiles at the Blue Grass Army Depot, Kentucky. In 2003, the preferred destruction method chosen by the Department of Defense (DOD) was neutralization of the material followed by supercritical water oxidation (SCWO).¹ The process that was chosen for GB destruction was chemical neutralization followed by secondary treatment; either oxidation (on-site) or biotreatment (transportation off-site). A toxicological assessment of potentially hazardous material is required prior to its transportation so that Department of Transportation (DOT) Hazard Classification and Packaging Categories may be assigned in the event that an accidental spill and subsequent exposure occur.

The Operational Toxicology Branch was tasked with testing the dermal hazards of pH adjusted neutralized GB hydrolysate (a caustic solution containing GB breakdown products) in rabbits in accordance with the Code of Federal Regulations (CFR) 49, Part 173.132² (DOT Guidelines). Similar studies on neutralized hydrolysates of mustard (HD)³ and VX⁴ were previously conducted by the Operational Toxicology Branch.

2. MATERIALS AND METHODS

2.1 Test Material.

On September 15, 2008, a sample of GB hydrolysate was obtained from the Environmental Toxicology Branch for testing. The Neutralized GB (hydrolysate) (GB/NaOH GB-8072) was produced using 7.5% GB (Chemical Agent Standard Analytical Reference Material grade, stabilized with tributylamine CAS# 102-82-9) in 6% NaOH. The sample was a clear golden brown color with very little precipitate. The pH of the sample was adjusted to 7.95 using 10% HCl with the final concentration of 92.5% of the original sample. This was done to assess the potential toxicity of the reaction products on the animals without excessively harming them due to the corrosive properties of the hydrolysate. The density of the hydrolysate was 1.043 g/mL.

2.2 Animals.

Fifteen New Zealand White (NZW) rabbits (8 male and 7 female) were procured from Millbrook Breeding Labs, Amherst, MA. The rabbits were requested in the weight range of 2.30-2.50 kg and arrived weighing 2.20-2.42. The animals had been ear tattooed by the vendor, but were randomly assigned a test number upon arrival. The rabbits were housed in large individual plastic cages inserted into stainless steel racks for a quarantine of 7 days. Cage waste collection pans were changed on a M-W-F schedule, while the floors were sanitized daily. The rabbits were fed a controlled diet of certified rabbit chow (Harlan Teklad, Madison, WI). Water

was provided *ad libitum*. The quarantine room temperature was $65^{\circ}\text{F} \pm 4^{\circ}$ with relative humidity (RH) of 40-60% and a 12 hr day/night light cycle. The testing facility was maintained at $75^{\circ}\text{F} \pm 2^{\circ}$ and RH at 40-60%.

2.3 Toxicity Testing.

Dermal testing began on September 16, 2008. The day before testing, 12 rabbits (6 male and 6 females) had the test area clipped free of hair. The test area was approximately 150 cm^2 from between the shoulders and rump and mid-way down the sides. On the day of testing, the animals were placed in the fume hood operating at $100\text{ Lpm} \pm 10\%$. A 2 layer gauze patch was applied to the rabbits back and secured with surgical tape to keep the test material in place. The test material (0.959 mL/kg) was gently applied to the animal's back and the gauze was used to help retain the liquid. Following compound deposition, the test site had a 6 mil polyethylene film placed over the area (semi-occluding) for 24 hr. After the 24 hr exposure, the gauze and polyethylene film were removed, the skin was rinsed with water and the test sites were blotted dry. The exposure site was evaluated for erythema and edema at 24, 48, and 72 hr, and 7 and 14 days. The animals were observed for toxic signs during this period. The DOT Hazard Classification and Packaging Categories for Division 6.1 Mixtures–Dermal Toxicity Guidelines were used (Table 1).

Table 1. DOT Hazard Classification and Packaging Categories for Division 6.1 Mixtures²

DOT Testing for Dermal Toxicity	
<u>Packing Group</u>	<u>Dermal Toxicity LD50(mg/kg)</u>
I	≤ 50
II	≥ 50 and < 200
III	≥ 200 and < 1000

The rabbits were evaluated for erythema and edema using the Dermal Irritation Scoring Procedures in Table 2.⁵

Table 2. Evaluation of Skin Reaction (Irritation)

	Value
Erythema and Eschar Formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum possible	4
Edema Formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges or area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible	4

3. RESULTS

Dermal irritation was observed in the majority of the rabbits tested (Table 3). The irritation was centered as white crusty papules with both edema and erythema scores ranging from 0-4. On visual inspection, it was thought that the irritation might have been from the tape used to secure the patch. However, it later became evident that the irritation was from the test material only.

Readings at 48 hr showed no change in erythema and a slight reduction in edema. Readings at 72 hr indicated the erythema had not changed. The edema showed significant reduction for some animals with readings of 3-4 (24 hr) to the lower score of 2 as the maximum at 72 hr. At 7 days, all of the erythema remained at scores of 4 except for rabbit # 7 who was completely healed. There was no edema in any of the rabbits at 7 days. Readings at 14 days post exposure showed significant reduction of the erythema; however, four animals still had the highest erythema score possible at 4. Since the skin still contained dry scaly and scabby areas, the erythema reading remained a 4. The scabby areas were healing well with pink skin and no other irritation of the site.

Table 3. Edema and Erythema Scores in Rabbits Following 24 Hr Contact with Neutralized GB Hydrolysate.

Section 1. Edema Scores						
Animal #	Sex	24 hr	48 hr	72 hr	7 day	14 day
1	F	0	0	0	0	0
2	F	0	0	0	0	0
4	F	2	2	1	0	0
5	F	0	0	0	0	0
6	M	4	3	2	0	0
7	M	2	2	1	0	0
8	M	2	2	2	0	0
9	M	1	2	2	0	0
10	M	2	2	2	0	0
11	M	3	1	1	0	0
12	F	4	3	2	0	0
13	F	4	1	1	0	0
Mean Scores for 24 and 72 hr		1.91		1.16		
Section 2. Erythema Scores						
Animal #	Sex	24 hr	48 hr	72 hr	7 day	14 day
1	F	0	0	0	0	0
2	F	0	0	0	0	0
4	F	4	4	4	0	0
5	F	0	0	0	0	0
6	M	4	4	4	4	4
7	M	4	4	4	0	0
8	M	4	4	4	4	4
9	M	4	4	4	4	4
10	M	4	4	4	4	0
11	M	4	4	4	4	0
12	F	4	4	4	4	4
13	F	4	4	4	4	4
Mean Scores for 24 and 72 hr		3		3		

Note: Primary Irritation Score based on 24 and 72 hr observations.
The Primary Irritation Score is $1.91 + 1.16 + 3 + 3/2 = 4.54$

4. DISCUSSION

The dermal toxicity testing with Neutralized GB hydrolysate did not produce any deaths or observable toxic signs in the 12 rabbits dosed with 0.959 mL/kg of the material. Therefore, it is not a "Class 6, Division 6.1 Poison", per DOT Regulations.²

It was observed that the test material did produce considerable dermal irritation in the form of erythema and edema. The dermal irritation lasted for 14 days. The Primary Irritation Score is 4.54, which places the neutralized GB hydrolysate into the moderate skin irritant category. It should be noted that a score of 5 would have placed the material into the Primary Irritant Category.

Table 3 is a summary of the edema and erythema scores for 1 (24 hr) to 14 days. The 7 day dermal observation indicated the edema (swelling) was completely gone. At 14 days, the erythema (scabby area) was healing nicely and all appeared that they would resolve back to normal skin.

5. CONCLUSIONS

The following conclusions were drawn from this test:

- The neutralized GB hydrolysate is not a "Class 6, Division 6.1 Poison."²
- The material is considered a moderate skin irritant with a score of 4.54.
- The 7 day dermal observations indicate the edema was completely gone; however, erythema was still present.
- At 14 days, the erythema remained a 4 (scabby areas) for four animals, but the other animals were healing well.

6. RECOMMENDATIONS

The neutralized GB hydrolysate should be considered as a potential primary dermal irritant due to the observed combined clinical signs and their severity and persistence (erythema). Appropriate personal protective equipment (butyl rubber gloves, aprons, safety face shield, and protective footwear) is highly recommended when a potential for dermal of this material exists.

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